IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPL. NO.	:	10/776,180
APPLICANT	:	Marc BEAUREGARD *et al.*
TITLE	:	METHOD OF MUTAGENIC CHAIN REACTION
FILED	:	02/12/2004
ART UNIT	:	1639
EXAMINER	:	Sue Xu Liu
Docket No.	:	15493-2US

DECLARATION OF MARC BEAUREGARD

I, Marc BEAUREGARD, do hereby declare and state as follows:

1. My academic background and experiences in the field of the present invention are listed on the enclosed *curriculum vitae*.
2. I am a Professor at Université du Québec à Trois-Rivières.
3. I am an author of several scholarly publications as listed in my enclosed *curriculum vitae*.
4. I am an inventor in the present application, I have read and am thoroughly familiar with the contents of U.S. Patent Application Serial No. 10/776,180, entitled "METHOD OF MUTAGENIC CHAIN REACTION", including the claims as originally filed and amended herewith.
5. I have also read and understood the latest Official Action from the PTO dated December 19, 2005, as well as the references cited therein. In this Office Action, certain claims were rejected for insufficient scope of enablement and failure to comply with the written description requirement under 35 U.S.C. §112, first paragraph.

6. Additional experiments performed under my supervision have shown that the mutagenic method enables the generation of mutants on three different templates. These experiments are described in **Exhibit A**.
7. Additional experiments have been performed by an independent laboratory under the supervision of Dr. Joelle Pelletier at Université de Montréal. Dr Pelletier used the mutagenic method to generate mutants of the *AxeA* gene. These experiments are described in **Exhibit B**.
8. **Exhibits A and B** clearly show that the mutagenic method successfully allowed the generation of mutant libraries irrespectively of the DNA sequence used as a template. The mutations presented therein possessed the same characteristics as those reported in the above-identified patent application.
9. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both (18 U.S.C. Sec. 1001), and may jeopardize the validity of the application of any patent issuing thereon.

Signed  Dated: June 5, 2006

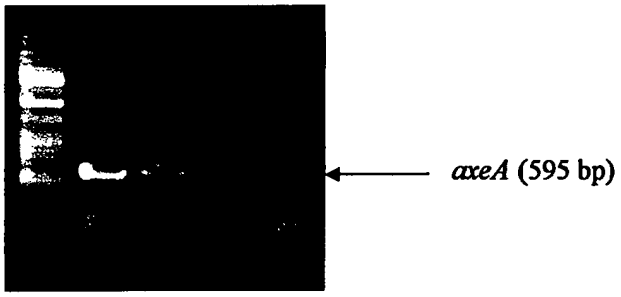
Marc BEAUREGARD

Exhibit A

Experiments previously performed under my supervision, using the conditions described in U.S. patent application 10/776,180 have shown that the mutagenic method is efficient to generate mutants of the *MB-1* gene (using microbial preferred codons), the *MB-1Trp* gene (using plant preferred codons), and with three restriction fragments obtained from the genome of *Actinobacillus pleuropneumoniae* 4079 (size: 0.8kb; 1.6kb; 2.8kb). The types of mutants generated are similar with respect to the range of mutation, the deletion frequency and the mutational bias when normalized to original content on A, T, C, or G.

Exhibit B

The gene *axeA* encoding a acetyl transferase and its catalytic domain protein sequence of the enzyme is GC-rich (68.8%). The gene was submitted to PCR amplification using the Vent® (exo-) DNA Polymerase (New England Biolabs), equimolar dNTPs and varying concentration of 1-propanol. The PCR conditions were as follows: 95°C 1min, 50°C 1min and 72°C 1min (35 cycles).



The 595 bp fragment amplification in presence of 1-propanol was average, and, an increase in alcohol concentration in the reaction mixture induced a decrease in amplification efficiency. Only amplification products B and C were further analyzed. They have been cloned in the vector used in the project before being introduced in *E. coli* SS320. Two DNA libraries were obtained.

Library sequencing was performed using a DNA Sequencer Long Readir 4200 (Licor). For preliminary studies, 10 clones were sequenced in each of the two libraries. For library B, 9 mutants out of 10 were analyzed and for library C, 7 mutants out of 10 were analyzed. With respect to library C, two identical clones were identified.

Table 1. Allocation of mutations observed in libraries B and C.

Libraries	A→C	A→G	A→T	C→G	C→T	C→A	G→C	G→T	G→A	T→A	T→C	T→G	Total mutations
B (5% propanol)		1	1	1	9	3		2	9		2		28
C (6% propanol)	2				3	2			2				9
Total mutations	2	1	1	1	12	5	0	2	11	0	2	0	37
% mutations	5,26	2,63	2,63	2,63	31,58	13,16	0,00	5,26	28,95	0,00	5,26	0,00	

The results indicated that mutations C→T et G→A represented more than 60% of the observed mutations. In addition, only one deletion has been observed for one clone in library B. For libraries B and C, 5 700 and 5 328 nucleotides have been sequenced respectively, giving a mutation frequency of 4.91×10^{3} and 1.69×10^{3} respectively.